Parathyroid Hormone Inhibits B Cell Proliferation: Implications in Chronic Renal Failure

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ABSTRACT

B cell proliferation is impaired in patients with chronic renal failure, but the mechanisms underlying this defect are not known. Lymphocytes have receptors for parathyroid hormone, and it is possible that the state of secondary hyperparathyroidism of renal failure is responsible for the B cell defect. Our studies were designed to (a) examine T cell-independent B cell proliferation (3H)thymidine incorporation) induced by Staphylococcus aureus Cowan I after 5 days of culture, (b) evaluate the effect of parathyroid hormone on S. aureus Cowan I-induced B cell proliferation, and (c) investigate the mechanisms through which parathyroid hormone may exert its effect on B cell proliferation. Lymphocytes were obtained from 37 normal subjects and 21 dialysis patients. S. aureus Cowan I induced significant stimulation (P < 0.01) of the proliferation of B cells from both groups, but the effect was smaller on B cells from dialysis patients (10.0 ± 1.4 x 10^3 cpm) than on those from normal subjects (21.8 ± 2.0 x 10^3 cpm). Both the intact molecule of parathyroid hormone (1-84 PTH) and its amino-terminal fragment (1-34 PTH) caused significant inhibition of proliferation of B cells from normal subjects in a dose-dependent manner, with the effect being significantly greater (P < 0.01) with an equimolar concentration of 1-84 PTH than that of 1-34 PTH. Inactivation of 1-84 PTH by oxidation abolished most of its inhibitory effect on B cell proliferation. The effect of 1-84 PTH was significantly greater (P < 0.05) on proliferation of B cells from normal subjects (–58.4 ± 2.5%) than on B cells from dialysis patients (–47.4 ± 3.9%). There was a significant (P < 0.01) inverse relationship between the blood levels of parathyroid hormone in the patients and the magnitude of the in vitro inhibition of B cell proliferation by parathyroid hormone. 1-84 PTH produced significant stimulation (P < 0.01) of cAMP production by B cells from normal subjects. Both forskolin and cholera toxin, agents that elevate levels of cAMP, also produced significant inhibition (P < 0.01) of S. aureus Cowan I-induced B cell proliferation. In contrast to the different effects of parathyroid hormone on the proliferation of B cells from normal subjects and dialysis patients, the actions of forskolin and cholera toxin on proliferation of B cells from these two groups were not different.

The results indicate that (a) B cells are a target for parathyroid hormone action, and these cells may have receptors for the hormone; (b) the inhibitory effect of parathyroid hormone on B cell proliferation is most likely mediated by the stimulation of cAMP production; and (c) the lesser effect of parathyroid hormone on the proliferation of B cells from dialysis patients could be due to desensitization and/or downregulation of parathyroid hormone receptors on B cells.

The results are consistent with the notion that the impaired proliferation of B cells in patients with chronic renal failure is, at least in part, due to the state of secondary hyperparathyroidism in these patients. Our data assign a new role for excess parathyroid hormone in the genesis of the uremic syndrome.

Key Words: Parathyroid hormone, B cells, chronic renal failure

Patients with chronic renal failure may display variable degrees of impaired humoral immunity (1). The antibody response to viral (2), but not bacterial, antigens (3) may be reduced, and the number of total B cells has been reported to be normal (4,5) or decreased (6–8). Further, Raskova et al. (5) found that in dialysis patients, both the T cell-dependent and T cell-independent B cell proliferation are reduced. The mechanisms underlying these abnormalities are not elucidated.

Available data indicate that lymphocytes have receptors for parathyroid hormone (PTH) (9–11). It is
therefore reasonable to suggest that the function of lymphocytes could be affected by PTH. Indeed, Perry (12) showed that 1-34 PTH activated mononuclear leukocytes, most likely T cells, and caused them to produce a substance(s) that enhances bone resorption. In addition, Atkinson et al. (13) reported that PTH stimulates thymic lymphocytes proliferation. Also, Klinger et al. (14) showed that both 1-34 PTH and 1-84 PTH produced significant stimulation of the phagocytin-induced T cell proliferation. It is, therefore, possible that PTH also affects the function of the B cells.

Patients with chronic renal failure have secondary hyperparathyroidism and elevated blood levels of PTH (15). The chronic exposure of the B cells to excess PTH in these patients may alter the ability of these cells to proliferate and to produce immunoglobulins in response to antigenic stimulation, and as such, may affect the humoral immunity in these patients.

This study examined the interaction between PTH and T cell-independent proliferation of B cells obtained from normal subjects and from dialysis patients and evaluated the potential mechanisms underlying such an interaction.

METHODS

Subjects

Twenty-one hemodialysis patients and 37 healthy subjects were studied. Among the dialysis patients, there were 10 females and 11 males; the ages ranged between 30 and 69 (50.8 ± 2.6) years, and the duration of the dialysis therapy ranged between 14 and 180 (68.6 ± 10) months. None of the patients had evidence of systemic diseases nor were they receiving medications known to affect the immune response. Among the healthy volunteers, there were 11 females and 26 males and the ages ranged between 24 and 39 (30 ± 0.7) years. The protocol of the study was approved by the Institutional Review Board of the Los Angeles University of Southern California Medical Center.

Separation of Peripheral Blood Mononuclear Cells (PBMC)

Peripheral venous blood was drawn under sterile conditions into vacutainers containing 20 U of preservative free heparin (GIBCO Laboratories, Grand Island, NY) per each 1.0 mL of blood. Blood samples from dialysis patients were collected before the dialysis procedure. PBMC were isolated from heparinized blood by density gradient centrifugation at 400 g for 30 min at room temperature on Ficoll-Hypaque solution (Isolymph, Gallard-Schlesinger, NY) and were washed and centrifuged twice (at 300 g for 10 min) with Hank’s balanced salt solution (HBSS) containing (per mL) 100 U of penicillin and 100 μg of streptomycin (GIBCO). After the final wash, cells were suspended at a concentration of 2 × 10⁶ cells per mL in growth medium RPMI 1640 (GIBCO) containing 10 mM HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid buffer and antibiotics [per mL] 100 U of penicillin and 100 μg of streptomycin) and supplemented with heat-inactivated 10% fetal calf serum (FCS) (Irvine Scientific, Irvine, CA) and 2 mM L-glutamine (GIBCO). This is referred to below as the culture media. Viable cell counts were performed by assessing trypan blue exclusion.

To determine the fraction of B cells in the PBMC preparation, 10⁵ cells were stained with fluorescein isothiocyanate-conjugated anti-CD20 monoclonal antibody (Leu 16; Becton Dickinson, Mountain View, CA). The number of positive cells was estimated by counting at least 100,000 cells with a FACSTAR flow cytometer (Becton Dickinson). The percentage of B cells in peripheral blood of normal subjects (10.0 ± 1.04%) was not different from that in dialysis patients (9.9 ± 0.45%).

Reagents

Parathyroid Hormone. Four preparations of PTH were used. These included the following: (a) bovine intact molecule of PTH (1-84 PTH) purchased from Sigma Chemical Co., St. Louis, MO; (b) synthetic amino-terminal fragment of PTH (1-34 PTH) purchased from Biochem, Torrance, CA; (c) inactivated 1-84 PTH; and (d) pure 1-84 PTH which was prepared by Dr. James Zull and E. Zull, Cleveland, OH. This moiety of the hormone was prepared from defatted parathyroid glands (Sigma). The hormone was extracted and purified with the final purification step, which included reversed phase high-pressure liquid chromatography, which removes oxidized hormone from the preparation. All high-pressure liquid chromatography separations were done by using Waters system and reversed phase C-18 Bondapak columns (7.8 × 300 mm). The solvent systems employed were water–acetonitrile–trifluoroacetic acid mixtures. The pure 1-84 PTH was assayed for its activity in the renal adenylate cyclase bioassay system. The inactivation of the hormone was done by oxidation as follows: 10 to 15 μg of 1-84 PTH was dissolved in 60 μL of 0.15 N acetic acid, and, to this solution, 40 μL of 30% (v/v) H₂O₂ was added. The solution was incubated at 37°C for 45 min, the reaction was terminated by freezing at −70°C, and the product was recovered by lyophilization. All preparations of PTH were dissolved in 100 mM acetic acid and diluted with culture media to obtain the desired concentra-
tions (1 × 10⁻⁷, 2 × 10⁻⁷, and 4 × 10⁻⁷ M for 1-84 PTH; 2 × 10⁻⁷, 4 × 10⁻⁷, and 8 × 10⁻⁷ M for 1-34 PTH; and 4 × 10⁻⁷ M for inactivated 1-84 PTH). All solutions of PTH were filtered for sterility through 0.22-μm-pore-size cellulose acetate membrane filters (Corning Glass Works, Corning, NY) and were used within 1 day.

Staphylococcus aureus Cowan I (SAC), SAC was obtained from Bethesda Research Laboratories, Inc., Gaithersburg, MD, was prepared by diluting whole formalinized bacteria with culture medium, and was used at a final concentration of 1:50,000 v/v, which was established as optimal for our culture conditions.

Forskolin, Dimethylsulfoxide, and Cholera Toxin. Stock solution of forskolin (Boehringer, San Diego, CA) was prepared in ethanol (5 mg/mL). Further dilution of forskolin was made in a complete culture medium to achieve the final concentration of 20 μg/mL. Cholera toxin (No. C-3012, Sigma) was dissolved in distilled water (0.5 mg/mL) and then diluted with the culture medium to obtain a final concentration of 0.1 μg/mL. A sample of 0.01 μL of these solutions was used in each well of the proliferation assay.

Proliferation Assay

The proliferation assay was made by the method described by Shenker and Matt (16). Lymphocytes were cultured for 5 days in flat-bottomed 96-well microplates (Linbro/Titerk; Flow Laboratories, McLean, VA) in a humidified environment of 37°C and 5% CO₂ in a water-jacket incubator (Forma Scientific, Marrietta, OH). Each well contained 2 × 10⁶ cells added to 0.1 mL of culture medium, 0.1 mL of SAC, and 0.05 mL of PTH preparation. Forskolin and cholera toxin were added in a volume of 10 μL. In cultures without PTH or mitogen, the desired amount of medium was added to reach the final volume of 250 μL. Cell proliferation was measured by the incorporation of [³H]thymidine (methyl-[^3]H)thymidine; specific activity, 6.7 Ci/mmol; ICN Radiochemicals, Irvine, CA), which was added in the amount of 0.5 μCi per well in 0.02 mL of medium for the last 4 h of culture. The cultures were then harvested onto glass-fiber filter paper by using an automatic cell harvester (Cambridge Technology Inc., Cambridge, MA). The filters were placed in scintillation vials, air-dried, and quenched in Betafluor (National Diagnostics, Manville, NJ) before being counted in a liquid scintillation counter (Model LS 7000; Beckman Instruments, Palo Alto, CA) for measurement of cell-bound radioactivity. Each study was performed in 3 to 4 replicates, and each well of these replicates was counted for 3 min; the result of each study is the mean cpm of the 3 to 4 replicates.

Effect of 1-84 PTH and Forskolin on cAMP Production

PBMC were isolated as described above. Cells (50 × 10⁶) were suspended in 25 mL of RPMI 1640 supplemented with 10% heat-inactivated FCS. A total of 0.1 mL of packed neuraminidase-treated sheep red blood cells was added to the cell suspension and incubated for 15 min at 37°C. The mixture was spun at 300 g for 5 min and then incubated on ice for 60 min. The mixture was resuspended by gentle rolling movements of the tube, and the cell suspension was underlayered with 15 mL of Isolymp. It was then spun at 400 g for 30 min. The cells were collected from the interface, washed twice with HBSS, and suspended in an adequate volume of RPMI with 10% FCS to give 5 × 10⁶ cells per mL. This cell suspension was incubated on petri plates (10 mL per plate) for 90 min at 37°C. The plates were rinsed three times with HBSS, and the washings were collected and spun at 300 g for 10 min. This separation procedure yielded a population containing 73 ± 2.4% of B cells as assessed after staining with fluorescein isothiocyanate-conjugated anti-CD20 monoclonal antibody (Leu 16; Becton Dickinson). However, the number of B cells recovered is about 4% of the total number of PBMC. Viability of the cells was ascertained with trypan blue exclusion and yielded over 95% viable cells.

The production of cAMP by these cells was measured. Cells (2 × 10⁶) were suspended in 1.0 mL of Krebs-Ringer solution with 10 mM HEPES–2.8 mM d-glucose–0.5 mg of BSA per mL (pH 7.4). A total of 25 μL of Krebs-Ringer, 1-84 PTH, or forskolin solutions was added to 0.5 mL of cell suspension, giving a final concentration of 4 × 10⁻⁷ M of 1-84 PTH and 20 μg of forskolin per mL, respectively. The cells were incubated in a water bath at 37°C for 30 min. The reaction was terminated by the addition of 240 μL of 10% perchloric acid. The mixture was then neutralized with 180 μL of 2 N potassium carbonate, diluted with 1.0 mL of distilled water, and centrifuged at 3,000 g for 8 min. The supernatant was saved, the pellet was washed and centrifuged twice, and the resulting supernatant was saved after each centrifugation. The pooled supernatant was dried in an oven (80°C) for 18 h and then dissolved in 2 mL of the cAMP assay buffer. cAMP was measured by using the cAMP (¹²⁵I) assay kit (Amersham Co., Arlington Heights, IL).

Measurement of PTH in Blood

PTH was measured by radioimmunoassay with sheep antisera 478 (kindly supplied by Dr. Claude Arnaud). ¹²⁵I-labeled bovine PTH, and pooled sera from patients with renal failure as standard. This antibody reacts predominantly with an immunologic
determinant in the carboxyl region of human PTH, and it will detect both the intact hormone and its carboxy-terminal fragment. The values for this assay in 63 normal subjects ranged from undetectable to 15 (5.7 ± 0.7) LEq/mL. The blood levels of PTH were detectable in 33 of the 63 (52%) normal subjects. Elevated blood levels of PTH were found in all of the 60 patients with chronic renal failure. The lower limit of detectability is 1 μLEq/mL.

Statistical Analysis

Student’s paired and unpaired t tests were used for statistical analysis. P values below 0.05 were considered significant. For nonparametric data, the Mann-Whitney U test was employed. For comparison of multiple group, multivariant analysis was made. Data are expressed as mean ± SE.

RESULTS

Available data indicate that SAC-induced lymphocyte proliferation represents T cell-independent B cell proliferation (17). Since, in all of our studies, we used SAC as a mitogen, we will refer to our data as those of B cell proliferation.

SAC induced a significant increase (P < 0.01) in proliferation of B cells of both normal subjects (from 2.4 × 10^3 ± [0.3 x 10^3] to 23.2 × 10^3 ± [2.1 x 10^3] cpm) and dialysis patients (from 2.2 × 10^3 ± [0.3 x 10^3] to 12.8 × 10^3 ± [1.5 x 10^3] cpm) (Figure 1). However, the increment in the dialysis patients (10.7 x 10^3 ± [1.4 x 10^3] cpm) was significantly lower (P < 0.01) than that in normal subjects (21.8 x 10^3 ± [2.0 x 10^3] cpm).

Both 1-34 PTH and 1-84 PTH produced a dose-dependent inhibition of SAC-induced B cell proliferation (Figure 2), but the effect of 1-84 PTH was significantly greater (P < 0.01) than that produced by an equimolar dose of 1-34 PTH. At a dose of 4 × 10^-7 M, the decrement in B cell proliferation was -9.7 ± 3.8% with 1-34 PTH and -55.2 ± 6.9% with 1-84 PTH (P < 0.01). Pure 1-84 PTH (4 x 10^-7 M) also significantly inhibited SAC-induced B cell proliferation from (9.7 x 10^3 ± 0.84 x 10^3) to (7.4 x 10^3 ± 0.69) cpm (P < 0.01 by paired t test). The magnitude of the inhibition (22.0 ± 3.6%) was significantly greater (P < 0.01) than that (8.9 ± 2.3%) induced by 4 x 10^-7 M 1-34 PTH (from 9.7 x 10^3 ± [0.84 x 10^3] to [8.9 x 10^3 ± [0.95 x 10^3] cpm; P < 0.05 by paired t test) (Figure 3).

Inactivation of 1-84 PTH abolished most, but not all, of the inhibitory effect of the hormone on the SAC-induced B cell proliferation. Proliferation of B cells from 14 normal subjects was (16.4 x 10^3 ± [1.5 x 10^3] cpm) with SAC alone, (7.8 x 10^3 ± [0.8 x 10^3]) cpm with SAC and 1-84 PTH, and (12.9 x 10^3 ± [1.4 x 10^3]) cpm with SAC and inactive hormone. The latter was significantly higher (P < 0.01) than that with SAC and active 1-84 PTH and significantly lower (P < 0.01) than that with the SAC alone.

1-84 PTH also inhibited SAC-induced proliferation of B cells from dialysis patients, but the decrement (-47.3 ± 3.9%) in the dialysis patients was significantly smaller (P < 0.05) than that in normal subjects (-58.4 ± 2.5%) (Figure 4). In 12 dialysis patients, the percent inhibition of SAC-induced B cell proliferation with 1-84 PTH was inversely and significantly cor-
Chronic Renal Failure, B Cells, and PTH

Figure 3. Effects of pure 1-84 PTH and 1-34 PTH on SAC-induced proliferation of B cells obtained from seven normal subjects. Each line represents one patient. Both pure 1-84 PTH and 1-34 PTH inhibited SAC-induced B cell proliferation, but the magnitude of the inhibition was significantly greater ($P < 0.01$) with pure 1-84 PTH than with 1-34 PTH.

related with the blood levels of PTH ($r = -0.78$, $P < 0.01$) (Figure 5).

Forskolin produced significant inhibition ($P < 0.01$) of SAC-induced B cell proliferation both in normal subjects ($-54.2 \pm 3.4\%$) and in dialysis patients ($-57.4 \pm 3.3\%$), and this effect was not different between the two groups. The percent inhibition in B cell proliferation produced by forskolin in normal subjects was not different from that produced by 1-84 PTH ($54.2 \pm 3.4$ versus $59.6 \pm 4.4\%$). However, the percent inhibition of B cell proliferation by 1-84 PTH in dialysis patients ($43.3 \pm 3.3\%$) was significantly lower ($P < 0.01$) than that produced by forskolin ($57.4 \pm 3.3\%$) (Table 1).

Cholera toxin also inhibited SAC-induced B cell proliferation both in normal subjects and in dialysis patients ($P < 0.01$). In normal subjects, the percent inhibition by cholera toxin was not different from that of 1-84 PTH but it was significantly higher ($P < 0.01$) than that produced by PTH in the dialysis patients ($40.4 \pm 8.7$ versus $13.7 \pm 5.7\%$) (Table 1).

Both $4 \times 10^{-7}$ M 1-84 PTH and forskolin significantly stimulated cAMP production by B cells ob-
TABLE 1. Effects of forskolin and cholera toxin on SAC-induced B cell proliferation in normal subjects and dialysis patients

<table>
<thead>
<tr>
<th>Studies of:</th>
<th>(^{(3)H})Thymidine uptake (cpm x 10^5)</th>
<th>% Inhibition in:</th>
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<tr>
<td></td>
<td>Normal subjects</td>
<td>Dialysis patients</td>
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<tr>
<td>Forskolin</td>
<td></td>
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<tr>
<td>SAC alone</td>
<td>23.7 ± 2.3</td>
<td>17.7 ± 2.6</td>
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<tr>
<td>SAC + PTH</td>
<td>9.3 ± 1.3^b</td>
<td>9.7 ± 1.3^b</td>
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<tr>
<td>SAC + Forskolin</td>
<td>10.8 ± 1.2^b</td>
<td>7.4 ± 1.1^b</td>
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<tr>
<td>Cholera toxin</td>
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<tr>
<td>SAC alone</td>
<td>14.0 ± 2.4</td>
<td>9.4 ± 2.3</td>
</tr>
<tr>
<td>SAC + PTH</td>
<td>8.7 ± 1.1^b</td>
<td>7.7 ± 1.3</td>
</tr>
<tr>
<td>SAC + Cholera toxin</td>
<td>8.3 ± 1.1^b</td>
<td>4.9 ± 0.3</td>
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</table>

^a The number of studies with forskolin was nine for both normal subjects and dialysis patients, and with cholera toxin was nine for normal subjects and five for dialysis patients. The dose of 1-84 PTH used in these studies was 4 x 10^-7 M.

^b P < 0.01 versus SAC (normal subjects and dialysis patients).

^c P < 0.01 versus SAC + PTH (dialysis patients).

^d P < 0.01 versus SAC + PTH in dialysis patients.

DISCUSSION

The results of this study show that both the intact molecule of PTH and its amino-terminal fragment inhibited in a dose-dependent manner the SAC-induced proliferation of B cells of normal subjects. However, the inhibitory effects of both 1-84 PTH from Sigma and of pure 1-84 PTH were significantly greater than that of the 1-34 fragment of the hormone. This latter finding is similar to that reported in other systems (14, 18-20). Indeed, 1-84 PTH exerted a greater effect on the beating of isolated heart cells (18), on fatty acid oxidation by skeletal muscle (19), on myocardial bioenergetics (20), and on phytohemagglutinin-induced T cell proliferation (14). These observations and those of this study are consistent with the notion that the intact molecule of PTH may attach more tightly to the hormone receptor or that other parts of the PTH, in addition to its amino-terminal fragment, may possess biologic activity.

Certain data exist supporting such possibilities. For example, binding sites with specificity for the middle region or the carboxy-terminal fragment of PTH have been described in canine and chicken renal membranes and in cloned rat osteosarcoma (21-23). Also, we have found that the carboxy-terminal fragment (19-84 PTH) exerts a biologic activity on human polymorphonuclear leukocytes in that it stimulates elastase release from these cells (24). Finally, our finding that inactivation of 1-84 PTH by oxidation did not completely abolish its inhibitory effect on B cell proliferation is in agreement with the suggestion that other parts of PTH beside the 1-34 fragment have biological activity since the oxidation of PTH by
H₂O₂ abolishes the biological activity of the 1-34 fragment of the hormone.

The dose of PTH used in our study is high and exceeds the amount of the hormone needed to exert its traditional actions on the kidney or bone. It is, therefore, appropriate to raise the question about the physiologic significance of the effect of this high dose of PTH on B cell proliferation. In recent years, many cells, other than the traditional ones, were found to be targets for PTH action and the in vitro dose of the hormone eliciting such nontraditional actions was also high. This is true in the case of heart cells (17), the hematopoietic system (25), red blood cells (26), polymorphonuclear leukocytes (24,27), T cells (14), pancreatic islets (28), and vascular smooth muscle (29). Potts (30) has considered this issue and discussed in detail various theoretical possibilities for this phenomenon. It is possible that these nontraditional actions of PTH are nonspecific and do not reflect hormone-receptors interactions. This does not seem likely, since PTH stimulated cAMP production by many of these cells including the heart (18), pancreatic islets (28), and T cells (14), as well as by B cells (as shown in this study). Further, the PTH effect on the pancreatic islets was blocked by its antagonist [Tyr-34]bPTH(7-34)NH₂ (28). All of these observations are consistent with the notion that these various cells may have receptors responsive to PTH. In addition, it is also possible that PTH elicits these nontraditional actions by stimulating cell receptors responsive to other agonists, i.e., PTH acts as a "surrogate for the normally active agonists" (30). In such a case, it is not surprising that one may find that different regions of the hormone, other than that (amino-terminal fragment) needed for its traditional effects on mineral metabolism, are responsible for the nontraditional actions. It is of interest that in many other cells, the amino-terminal fragment of PTH did not elicit the nontraditional effects. If PTH indeed acts as a surrogate for other active agonists, one would expect that a higher dose of the hormone is needed to mediate a biological action. Also, one should consider the possibility that the nontraditional actions of PTH are mediated by the activation of specific receptors that may or may not be related to the PTH receptors responsible for the mineral effects of the hormone. Such potential PTH receptors responsible for its nontraditional effects may require higher amounts of PTH for their activation. Finally, the in vitro cell preparation may contain cell components, such as proteases, which may degrade the hormone and, hence, higher amounts are needed to elicit its effects. These various possibilities have not been addressed by our studies.

The response of B cells obtained from dialysis patients to PTH was qualitatively similar but quantitatively different from that of B cells from normal subjects. Indeed, the SAC-induced proliferation of B cells from dialysis patients was significantly less than that of B cells from normal subjects. This finding is similar to that reported by Raskova et al. (5). It is theoretically possible that this difference is due to differences in the percentage of B cells in the PBMC and/or in the age between the normal subjects and the dialysis patients. Both of these possibilities seem unlikely. First, there was no significant difference between the percentage of B cells in the PBMC of both groups. Second, in five normal subjects and five dialysis patients matched in age (36.8 ± 1.28 versus 37.6 ± 2.13 years), SAC-induced B cell proliferation increased from (2.4 ± 10⁵) ± (0.29 ± 10⁵) to (31.9 ± 10⁵) ± (2.7 ± 10⁵) cpm and from (2.7 ± 10³) ± (0.26 ± 10³) to (14.8 ± 10³) ± (2.9 ± 10³) cpm, respectively (P < 0.01). Further, there was no correlation between age and SAC-induced B cell proliferation in both groups. Also, 1-84 PTH caused a smaller inhibition of proliferation of B cells from patients than from normal subjects. These observations indicate that the chronic exposure of the B cells to excess PTH in uremic patients is associated with reduced responsiveness to exogenous PTH, and such a resistance may be modulated by the degree of secondary hyperparathyroidism. Our finding that the percent inhibition of SAC-induced proliferation of B cells from dialysis patients was inversely correlated with blood levels of PTH supports such a postulate. A corollary to such a phenomenon has been documented in another organ. Patients with renal failure and those treated with dialysis display a skeletal resistance to the calcemic action of PTH (31,32), and this resistance has been attributed to desensitization or down-regulation of PTH receptors in bone as a consequence of the elevation of blood levels of PTH (33). It is possible that a similar phenomenon may be operative in the interaction between PTH and B cells in uremia.

To our knowledge, there are no studies demonstrating PTH receptors on B cells from normal subjects. However, Bialasiewicz et al. (34) reported that B cells from patients with Hodgkin's disease, acute leukemia, Burkitt's lymphoma, and thymic hyperplasia kept in culture had receptors for bovine PTH. Also, data are available consistent with the presence of PTH receptors on lymphocytes (9–11). Recently, Yamamoto et al. (11) demonstrated that the binding of PTH to circulating bovine lymphocytes satisfies the criteria for specific binding between the hormone and its receptors and they defined the properties of this receptor. Their properties correlated well with those reported for PTH receptors of renal and osseous origin (35–38). It is, therefore, reasonable to assume that PTH receptors are present on both T and B cells. Indeed, previous studies have shown that PTH affects the function of T cells, documenting an interaction between these cells and the hormone (12–14). The
results of this study showed that PTH exerts an effect on B cells as well and, therefore, are consistent with the presence of a receptor for PTH on B cells.

Several observations suggest that cAMP may inhibit B cell function and interfere with its response to mitogens. First, Shenker and Matt (16) found that forskolin inhibited SAC-induced B cell proliferation. Second, Hoffman (40) showed that cAMP inhibits mouse B cell proliferation, which is facilitated by B cell-stimulating factor 1. Third, Shearer et al. (41) reported that cAMP caused a significant inhibition of immunoglobulin secretion by human lymphoblastoid B cell line. Fourth, our data also showed that cAMP-elevating agents, such as forskolin and cholera toxin, inhibited proliferation of B cells from normal subjects and dialysis patients.

It is possible, therefore, that PTH stimulates cAMP production through its interaction with its receptors on B cells. Such an event could, at least in part, be responsible for the inhibitory effect of PTH on SAC-induced B cell proliferation. Indeed, our results show that 1-84 PTH stimulated cAMP production by B cells and both forskolin and cholera toxin—agents known to elevate intracellular cAMP—produced inhibition of the proliferation of normal B cells comparable in magnitude with that induced by PTH.

It is of interest that the effect of PTH was similar to that of forskolin and cholera toxin on B cells from normal subjects but significantly less on B cells from dialysis patients. It should be mentioned that the increase in cAMP production by forskolin and cholera toxin does not require receptor interaction but that induced by PTH does. It could be argued, therefore, that a downregulation or desensitization of the PTH receptors on B cells in the dialysis patients due to prolonged exposure to high levels of PTH in blood is associated with less production of cAMP by PTH than by forskolin and cholera toxin. Such a phenomenon may explain the differences in the action of PTH and forskolin or cholera toxin on the proliferation of B cells of the dialysis patients.

The results of our study are consistent with the proposition that the state of secondary hyperparathyroidism of chronic renal failure may play an important role in the genesis of the deranged humoral immunity in uremia. The inhibition of B cell proliferation by PTH may also lead to impaired antibody production by these cells. Indeed, preliminary studies from our laboratory showed that PTH inhibits immunoglobulin production by cultured B cells obtained from normal subjects (42).

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REFERENCES

18. Bogin E, Massry SG, Harary I: Effect of parathyroid


